REMARKS

These remarks are responsive to the final Office action dated July 5, 2006, and support the accompanying Request for Continued Examination as a submission under 37 C.F.R. § 1.114(c). Claims 50-59 and 61-66 are pending in the application. In the Office action, the Examiner rejected all of the pending claims as being obvious under 35 U.S.C. § 103(a):

- Claims 50-52, 54, 55, 57, 59, and 62-65 were rejected over U.S. Patent No. 6,287,774 to Nikiforov ("Nikiforov") in view of Zhou, et al. "Detection and Sequencing of Phosphopeptides Affinity Bound to Immobilized Metal Ion Beads by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry", *J. Am. Soc. Mass Spectrom.*, 2000, 11, pp. 273-282 ("Zhou");

- Claim 56 was rejected over Nikiforov in view of Zhou and further in view of U.S. Patent No. 6,022,708 to de Sauvage et al.;

- Claims 53, 58, and 66 were rejected over Nikiforov in view of Zhou and further in view of U.S. Patent No. 5,424,190 to Fuller; and

- Claim 61 was rejected over Nikiforov in view of Zhou and further in view of U.S. Patent No. 5,776,487 to Maxfield Wilson et al.

Applicants traverse the rejections. Applicants contend that the rejected claims are not obvious. In support of this contention, applicants previously provided objective evidence showing that the claimed invention offers unexpected benefits, which serve as secondary indications of nonobviousness. The following remarks (1) reiterate the unexpected benefits, (2) cite Patent Office policy regarding the Examiner's obligation to weigh the unexpected benefits against any indications of *prima facie* obviousness offered by the cited references, and (3) explain why one of the unexpected benefits, an increase in luminescence intensity upon binding to gallium relative to quenching of luminescence intensity upon binding to iron, is not expected and overwhelmingly outweighs any assertion of obviousness, to provide patentability of the claimed

invention. Accordingly, in view of the following remarks, applicants respectfully request reconsideration of the rejected claims, and prompt issuance of a Notice of Allowability.

I. Request for Continued Examination

Applicants are submitting herewith a Request for Continued Examination (RCE) under 37 C.F.R. § 1.114. This Request complies with the requirements of 37 C.F.R. § 1.114. In particular:

(i) Prosecution in the application is closed, since the last Office action was a final Office action under 37 C.F.R. § 1.113.

(ii) The Request is accompanied by a submission as set forth at 37 C.F.R. § 1.114(c), specifically, the remarks set forth herein.

(iii) The Request is accompanied by the fee set forth at 37 C.F.R. § 1.17(e).

Accordingly, applicants respectfully request grant of their Request for Continued Examination.

II. Rejections under 35 U.S.C. § 103

In the Office action, the Examiner rejected claims 50-59 and 61-66 under 35 U.S.C. § 103(a) as being unpatentable over Nikiforov in view of Zhou (claims 50-52, 54, 55, 57, 59, and 62-65) or further in view of an additional reference (claims 53, 56, 58, 61, and 66). With regard to the only independent claim, claim 50, the Examiner suggested that Nikiforov teaches all of the elements of claim 50, with the exception that Nikiforov discloses a binding partner including iron instead of gallium.[1] In addition, the Examiner stated that Zhou provides the motivation to replace iron with gallium."[2] Applicants traverse the rejections. The claimed invention is patentable over the cited references because the claimed invention provides unexpected benefits, documented

[1] Office action (July 5, 2006) pg. 2, second-to-last full paragraph, to pg. 3, second full paragraph.
[2] Id. at pg. 3, third full paragraph.

as objective evidence by applicants, that rebut the *prima facie* case of obviousness asserted by the Examiner.

III. Objective Evidence of Nonobviousness

Obviousness is a question of law based on analysis of four "Graham factors": (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art; and (4) objective evidence of nonobviousness.[3] The U.S. Patent and Trademark Office has a stated policy to follow *Graham v. John Deere Co.* in the consideration and determination of obviousness under 35 U.S.C. § 103.[4]

The Manual of Patent Examining Procedure is unequivocal with regard to the importance of the fourth Graham factor, objective evidence:[5]

> **Objective evidence or secondary considerations such as unexpected results**, commercial success, long-felt need, failure of others, copying by others, licensing, and skepticism of experts are relevant to the issue of obviousness and **must be considered in every case in which they are present**. When evidence of any of these secondary considerations is submitted, the examiner must evaluate the evidence. (emphasis added by applicants)

Moreover, the Manual of Patent Examining Procedure obligates the Examiner to compare evidence of unexpected results with evidence of *prima facie* obviousness:[6]

> "Evidence of unexpected results must be **weighed** against evidence supporting *prima facie* obviousness in making a

[3] *Graham v. John Deere Co.*, 383 U.S. 1, 17, 148 USPQ 459, 467 (1966).
[4] See MPEP at § 2141(I).
[5] See Id. at § 2141(III).
[6] See Id. at § 716.02(c)(I).

final determination of the obviousness of the claimed invention." (emphasis added by applicants)

In other words, evidence of *prima facie* obviousness can be outweighed by evidence of unexpected results, to render a claimed invention nonobvious and patentable. Accordingly, the claimed invention may be nonobvious and patentable, based on strong objective evidence of unexpected results, even though the prior art is alleged to meet every requirement of a *prima facie* case of obviousness: (1) some suggestion or motivation to combine reference teachings, (2) a reasonable expectation of success, and (3) a teaching or suggestion for all the claim limitations.[7]

IV. Reiteration of Unexpected Benefits

Applicants have found at least three significant unexpected benefits to using gallium in luminescence polarization assays that are neither taught nor suggested by Nikiforov, Zhou, or any other prior art reference of record. The unexpected benefits were presented as objective evidence of nonobviousness[8] in a Declaration from one of the inventors included in the previous response to Office action.[9] In brief, the unexpected benefits are:

- First, gallium enhances intensity, instead of quenching intensity like iron, after associating with luminescent assay components. Consequently, assays employing gallium can be performed much more quickly and with much less statistical noise than assays employing iron. For example, a gallium reagent tested by applicants, relative to an iron reagent, permitted detectable emission of about one-hundred fold more light from a bound, exemplary luminophore.

[7] See Id. at § 2143.
[8] See Id. at §716.01(a).
[9] Response to Office Action (April 4, 2006), Exhibit A.

- Second, assays employing gallium, in contrast to assays employing iron, have a much greater dynamic range of polarization. Consequently, assays employing gallium are much more robust and easy to perform than assays employing iron, if the latter can be performed at all. For example, a gallium reagent tested by applicants had a large, near maximal dynamic range of polarization, while an iron reagent had a small, near minimal dynamic range of polarization.

- Third, assays employing gallium to bind product can better distinguish the existence of product in a mixture of substrate and product, relative to iron, since product bound to gallium will contribute more rather than less to the total polarization.

Overall, the use of the gallium reagent in place of the iron reagent converts an effectively unusable polarization assay into one of high sensitivity and robust performance.

None of the references of record, including Nikiforov and Zhou, teaches or suggests any of these unexpected and patentable benefits of using a binding partner including gallium in polarization assays.

V. Examiner's Response to Applicants' Unexpected Results

In the Office action, the Examiner was not persuaded by the unexpected results provided by applicants. More particularly, the Examiner responded to applicants' arguments about unexpected results with two assertions. First, the Examiner asserted that there is a motivation to combine the references:[10]

> These arguments however are not persuasive because Nikiforov teach that the metal in general is a multivalent metal cation that may for example be Fe^{3+} (see col. 13, lines 32-39.) Zhou et al. specifically teaches gallium, i.e., Ga^{3+}, which is a multivalent metal cation and that Ga^{3+} has better selectivity for the phosphopeptides than iron (page 274, left column, last paragraph), which provides the

[10] See Office action (July 5, 2006) pg. 11, lines 14-21.

> motivation to use Ga^{3+} in the Nikiforov invention as it would facilitate the detection of phosphorylated peptides because of its better selectivity for phosphorylated peptides, which would result in more accurate results.

Second, the Examiner asserted that the unexpected results offered by applicants are expected from the teachings of the prior art:[11]

> Based on the teachings of Zhou et al., it would be **expected** that Ga^{3+} would more readily facilitate the assay and provide more sensitive or clearer results than using iron, Fe^{3+}, **as would be indicated by higher intensity**, because Zhou et al. teach that Ga^{3+} has better selectivity for the phosphopeptides than Fe^{3+}. (emphasis added by applicants)

VI. Applicants' Rebuttal to the Examiner's Response

A. Overview

Applicants maintain that any alleged motivation to combine Nikiforov and Zhou (the first assertion quoted above in Section V) relates to the apparent strength of a *prima facie* case of obviousness. As outlined above in Section III, indications of *prima facie* obviousness should be weighed against the strength of the unexpected results. Applicants strongly disagree with the Examiner's position (the second assertion quoted above in Section V) that any of the benefits of gallium presented by applicants would have been expected based on the teachings of Zhou. In particular, applicants believe that nothing in Zhou even arguably teaches or suggests that gallium has an ability to provide any of the above-identified benefits: (1) enhanced luminescence intensity instead of quenching, (2) a much greater dynamic range of polarization, or (3) more sensitive recognition of product in a polarization assay. However, to simplify the

[11] See Office action (July 5, 2006) pg. 11, line 21, to pg. 12, line 2.

following discussion, applicants will focus below on the first-listed unexpected benefit (enhanced luminescence instead of quenching), which alone should be sufficient to outweigh any assertion of *prima facie* obviousness.

B. Zhou

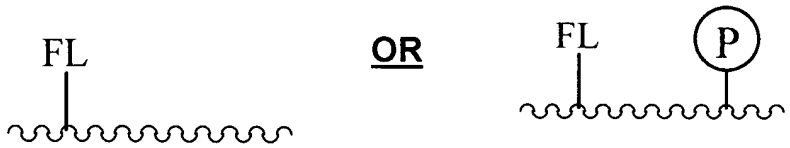
Zhou does not teach or suggest any difference in the luminescence properties of iron and gallium (since Zhou does not even mention luminescence at all). For example, Zhou does not disclose any difference in the ability of iron and gallium to affect luminescence intensity when associated with a luminophore. Nevertheless, the Examiner cited an alleged "better selectivity" of gallium over iron to counter applicants' assertion of unexpected benefits of gallium over iron. However, the "better selectivity" of gallium does not have any apparent relationship to luminescence properties and thus, independent of whether it is relevant to a binding assay, cannot and does not provide any expectation about the luminescence properties of either gallium or iron. Therefore, based on the teaching of Zhou, and iron's known behavior as a luminescence quencher, one of skill in the art would not have expected gallium to show less (or more) quenching of luminescence intensity than iron when associated with a luminophore.

C. Comparison of Actual and Expected Luminescence Intensities

The Examiner suggested that the "better selectivity" of gallium over iron for phosphopeptides provided an expectation (from Zhou) of a higher intensity of luminescence when gallium, instead of iron, is used in a binding assay with a luminescent phosphopeptide. Applicants disagree strongly for the reasons forth in the remainder of this subsection.

Applicants previously presented a Declaration with experimental data from binding assays comparing reagents that include iron ("iron reagent") or gallium ("gallium

reagent").[12] Each binding assay included either a fluorescent ("FL") substrate (a luminophore-labeled, nonphosphorylated peptide; "S") or a fluorescent product (a phosphorylated form of the peptide; "P"), as follows:



"Substrate (S)" **"Product (P)"**

(The product corresponds to a product that would be produced by operation of a suitable kinase enzyme on the substrate.) The fluorescent substrate or product was incubated with the iron or gallium reagent. Then, the effect of each metal on luminescence intensity was measured. Accordingly, these binding assays with gallium correspond to an embodiment of the invention for detecting kinase enzyme activity in which there is no enzyme activity (substrate (S) only) or substantial enzyme activity (product (P) only).

Figure 1 of the Declaration, which is reproduced below as the graph on the left,[13] shows actual results of total luminescence intensity measurements, in relative fluorescence units (RFU), as a function of added metal (iron (Fe) or gallium (Ga)) and peptide phosphorylation state (P or S) in the binding assays. The luminescence intensity (or brightness) generally was comparable in assays of the substrate (S) performed with either iron or gallium. These results are consistent with little or no binding of each metal to this nonphosphorylated substrate, and thus little or no effect on

[12] Response to Office Action (April 4, 2006), Exhibit A.
[13] The graph has been modified slightly relative to the Declaration by addition of an asterisk and a pair of lead lines extending from the asterisk.

the intensity of the luminophore associated with this substrate. In contrast, the luminescence intensity (or brightness) differed dramatically according to which of these reagents was present in assays of the product (P (luminescent phosphopeptide)). In particular, the intensity was about one-hundred-fold higher in assays of the product performed with gallium relative to assays of the product performed with iron. This dramatic difference in intensity was produced, in part, by an approximately four-fold increase in the intensity of the product relative to substrate (S) for gallium, and, in part, by a more than twenty-fold decrease in the intensity of the product relative to substrate for iron. Therefore, in contrast to iron, which quenched luminescence intensity substantially when present with luminophore-labeled product, gallium not only did not quench but actually enhanced the intensity of luminescence from the product. The increase in luminescence intensity provides substantial benefits to the claimed invention, as described further in Subsection D below.

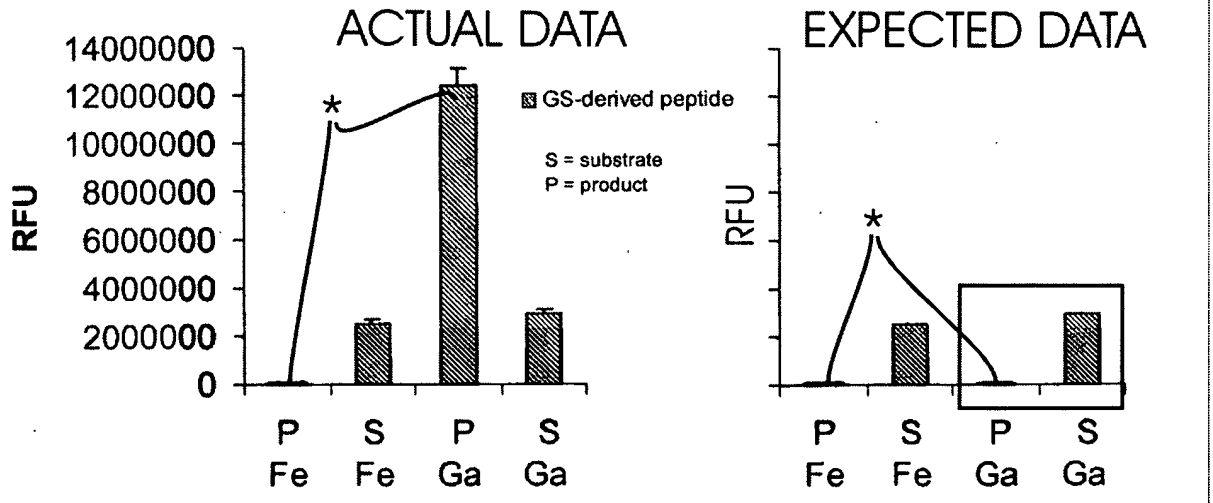


Figure 1 (Declaration) Expected Results with Gallium

These actual results, obtained by applicants, are compared above with a graph of expected results for gallium, shown to the right of the actual results. In the graph on

the right, the actual results for iron from applicants are compared with the expected results for gallium (shown boxed), based on Zhou. The dramatic difference between actual and expected results is indicated by the asterisks and paired lead lines extending therefrom, and demonstrates that the actual results with gallium are very unexpected.

The Examiner asserted that Zhou teaches more selective binding of gallium to phosphopeptides. Applicants disagree, because the selective "binding" of Zhou relates not only to binding itself, but instead, to a combination of engagement and release. However, only for the sake of argument, if Zhou did teach greater selectivity for binding of gallium for phosphopeptides, one of skill in the art would have expected gallium to bind more than iron to the product (P) (and/or less than iron to the substrate). As a result, the luminescence intensity of gallium with product (P/Ga) would have been expected to be less than (or about the same as), the product/iron (P/Fe) signal, because more binding would have been expected to produce even more quenching and less intensity (or about the same amount of binding and about the same quenching/intensity). Instead, the luminescence intensity of the product bound to gallium is at least about one-hundred fold higher than expected. "Better selectivity" cannot and does not explain this unexpected result because better selectivity of gallium relative to iron would not have been expected to counteract the expected negative effect of substantial quenching of either metal when bound to luminophore-labeled product.

The expected result based on Zhou, marked above by an asterisk in the graph on the right, is dramatically different than the actual unexpected result from applicants, marked by the asterisk in the graph on the left. Furthermore, even if the Examiner were to assert that one of skill in the art would have expected gallium and iron to have some

measurable difference in their effects on luminescence intensity, there is no teaching or suggestion about the direction or magnitude of this difference.

D. ***Importance of the Unexpected Benefit of Increased Luminescence Intensity***

The experimental results presented in the Declaration[14] and summarized above show that gallium provides a dramatic and unexpected increase in luminescence intensity relative to iron, when measured from metal-bound, luminophore-labeled peptide. Importantly, measurements of luminescence intensity underlie all measurements of luminescence polarization,[15] so the increased luminescence intensity is an unexpected benefit of gallium in the claimed invention.

The dramatic differences in intensity between iron and gallium observed by applicants translate into dramatic differences in the timing and sensitivity of assays performed with these reagents. Polarization assays on large numbers of samples are configured to be analyzed as fast as possible, to maximize throughput. For example, the SmartRead™ system employed in polarization readers produced by Molecular Devices Corporation is designed to collect data on each sample until a threshold number of photons (or a timeout period) has been reached, and then move on to the next sample. This threshold number of photons typically is selected to correspond to a minimally

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[14] Response to Office Action (April 4, 2006), Exhibit A.

[15] The relationship between polarization and intensity is expressed by the following equation:

$$P = \frac{I_{||} - I_{\perp}}{I_{||} + I_{\perp}}$$

Here, P is the polarization, $I_{||}$ is the intensity of luminescence polarized parallel to the polarization of the excitation light, and $I_{\perp}$ is the intensity of luminescence polarized perpendicular to the polarization of the excitation light, all following excitation with polarized light. Thus, measurements of polarization are only as good as the underlying measurements of intensity. See, e.g., Application, pg. 59, line 20, to pg. 60, line 4.

acceptable signal-to-noise ratio for data analysis.[16] Thus, due to the opposing effects of iron and gallium on brightness, it would take about 100 times as long to collect comparable light per sample with the iron reagent as with the gallium reagent. This difference literally is the difference between practical and impractical in high-throughput drug screening, since assay measurements that take 20-40 milliseconds to perform with gallium would take a completely unacceptable 2-4 seconds to perform with iron! Moreover, in any context, this difference means that for a given measurement time the signal strength (and thus the signal-to-noise ratio) will be significantly higher in gallium-based polarization assays than in iron-based polarization assays.

The dramatic intensity advantages of gallium relative to iron are not obvious or expected. To the contrary, metals are well-known luminescence quenchers (i.e., extinguishers). Indeed, Pierce Biotechnology sells a kinase assay system in which enzyme activity is observed using luminescence quenching that accompanies interaction of a fluorescently labeled phosphorylated peptide with iron.[17] Specifically, in the Pierce assay, luminescence intensity decreases monotonically with increasing phosphorylation, increasing kinase concentration, and increasing time, all reflecting increasing association of iron with the luminophore.[18] In contrast, applicants have discovered that gallium, unlike iron, not only does not quench but instead actually enhances intensity when bound to a luminophore. None of the references of record, including Nikiforov and Zhou, teach or suggest this unexpected and patentable benefit of using a binding partner including gallium in polarization assays.

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[16] The signal-to-noise ratio of the intensity in photon processes is proportional to the square root of the number of photons collected.

[17] See, e.g., Pierce IQ® Assay Platform: Technical Handbook (Pierce Pub. No. 1600963) (August 2003). This handbook is included with the Response to Office Action (April 4, 2006) as Exhibit B.

[18] Id.

In summary, the unexpected benefit of increased luminescence intensity provided by gallium far outweighs any assertion of *prima facie* obviousness and any expected benefit of gallium that might be derived from the teachings of Zhou. In particular, without this unexpected benefit, any expected benefit from increased selectivity of gallium allegedly taught by Zhou would still be dominated by the expected quenching by gallium. The lack of quenching by gallium, as shown by applicants, which provides an unexpected benefit of increased luminescence, thus both enables the claimed assay and rebuts any assertion of *prima facie* obviousness by the Examiner. Therefore, for at least these reasons, applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 103(a) and prompt allowance of the pending claims.

VII. Conclusion

Applicants believe that this communication is fully responsive to the Office Action, and that the claims are currently in condition for allowance. However, if there are any remaining matters, or if it would otherwise advance prosecution of the application, the Examiner is encouraged to call the undersigned attorney at (503) 224-6655.

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on January 5, 2007.

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Respectfully submitted,

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